

Amendments to the Specification:

Please make the following amendments to the specification by replacing the identified paragraphs below.

At page 24, line 5:

Notably, typical long PCR reagents are not employed in the methods of this invention. That is, a proofreading polymerase is not employed, and the polymerase used is much less processive than a polymerase used in long PCR. Typically, it is present at an activity of about [1~~u~~ μ] whereas that of long PCR is [2-5~~u~~ μ]. Other reagents such as DMSO and glycerol used primarily to stabilize the polymerases used in long PCR are undesirable in the short PCR methods practiced in this invention. Further, TRIS buffer is preferred in the short PCR practiced with the inventive methods herein whereas TRICINE buffer would otherwise be used with long PCR techniques.

At page 30, line 1:

PCR MasterMix

{Mg ⁺⁺ }	(as MgCl ₂), 3mM (except for Example 1, which was 4.5 mM); from Perkin-Elmer
{dNTP}	0.2 mM each, from Boehringer-Mannheim
{Taq pol}	1u/reaction well of ~ 50 ul
{beacon}	0.2 uM (except for Examples 2 & 3, where it was 0.083 uM)
{each primer}	0.15 uM
{buffer}	10 mM Tris-HCl, pH 8.3, 50 mM KCl; no internal ROX standard dye; (obtained commercially from Perkin-Elmer).

Restriction enzymes ScaI and PstI were purchased from New England BioLabs. Specimens were initially digested in restriction-specific buffers supplied by the vendor using 1 μ l of enzyme/ μ g of DNA as measured spectrophotometrically (except for PstI, where 15 μ l of 1 μ g of DNA was used). Reaction mixtures were incubated at 37 degrees C for 1 hour, then heat inactivated at 80 degrees C for 20 minutes. In all cases, 2 μ l of digest were used per PCR well.

DdeI was purchased from Gibco. Two μ l of enzyme were used to restrict 4 μ l of mt-DNA at a concentration of 0.24 μ g/ μ l, diluted into 4 μ l of 10X buffer diluted with 30 μ l of deionized water. Incubation was at 37 degrees C for 3.5 hours.

At page 33, line 27:

Master mix:

{Buffer} 10 mM Tris-HCl, pH 8.3, 50 mM KCl from Perkin-Elmer
{MgCl₂} 5 mM from Sigma
{dNTP, each} 0.2 mM from Kodak
{Taq pol} 1u/reaction well (50 μ l)
{5Mb3-TET (beacon)} 0.1 mM (if used)
{Primer, each} 0.17 μ M

At page 34, line 6:

The restriction enzyme Hind III was purchased from New England BioLabs. The suggested standard reaction conditions were used, except with approximately 5U of enzyme per μ g of DNA, instead of 1 U/ μ g.

At page 38, line 1:

The calibration equation is:

$C_t = \text{Intercept} + \text{Slope} \times \log \{\text{copy \#}\}$

When the copy # = 1, the $\log \{1\} = 0$; hence,

C_t for a single copy is given by the intercept; hence the intercept indicates the number of PCR cycles required for single copy sensitivity.

At page 40, line 3:

The PCR mastermix consisted of :

10 mM TRIS, pH

5 mM MgCl_2 ,

50 mM KCl;

0.2 mM, each dNTP,

$\sim 2 \text{ } \mu\text{g}$ of Taq DNA polymerase

At page 42, line 19:

Although PleI and ScaI can be used for restricting wt DNA as described in earlier examples, it is convenient to use just one restriction enzyme capable of cleaving both the 7 kb and 5 kb wt mt-DNA within their respective deletion sequences. Hind III , which cuts the following sequence:

AAGCTT

TTCGAA,

is used for this purpose. It does not cut the control region; hence, when it is desired to quantitate deletions relative to the control region, Hind III is especially preferred. It is incubated for 1 hour at 37 C with DNA at a level of $\sim 1 \text{ } \mu\text{g}$ μg DNA, as described by the manufacturer (New England Nuclear).